**Supplementary Tables and Figures**

**Supplementary Table 1. Mutation status of bladder cancer cell lines.** Grey indicates presence of alterations in these genes. Specific mutations in the PI3KCA gene are indicated.

**Supplementary Table 2. Synergy of TAK-228 in combination with TAK-117 determined by combination index**. Three independent assays were performed for each cell line. Cells were treated with the drugs at their equipotent ratio (e.g., at the ratio of their IC50s). After the ratio was set, we produced a mixture of the 2 drugs at 4 times their IC50 and serially diluted the mixture (e.g. 4-fold dilutions to 2-fold, 1-fold, 0.5-fold, 0.25-fold and 0.125-fold) to obtain a good dosage range and dose density. Combination index (CI) values for RT4, CAL-29 and T24 were calculated employing Calcusyn software. It utilizes the method applied by Chou and Talalay for formal synergy analysis and a computerized simulations determines the CI to quantitatively define synergism (CI<1), additive effect (CI=1), and antagonism (CI>1).

**Supplementary Figure S1. Cellular effects of TAK-228 on human bladder cancer** **cell lines. A. Effects of TAK-228 on apoptosis.** Cells were seeded in 100mm2 dishes and allowed to adhere overnight. Cells were treated with TAK-228 [50nM] for 24 and 48 hours and apoptosis was analysed by the Annexin V and Dead Cell Assay Kit (Millipore). Representative flow cytometry dot plots of 2 independent experiments are shown.Difi cells treated with cetuximab [10µg/ml] (CTX) were used as positive control. Western blot was performed on total cell lysates from the cells treated with TAK-228 [50nM or 200nM] for 24, 48 and 72h. cleaved-PARP antibody was used as a marker of apoptotic cells. Quantification of the results is shown; these were performed as for Figure 1. **B. Effects of TAK-228 on autophagy.** For western blot experiments, CAL-29 cell line was processed, treated and results evaluated as in Figure 1C.Representative images of 3 separate experiments are shown.

**Supplementary Figure S2. Molecular effects of everolimus** on the PI3K/mTOR signaling pathway **on bladder cancer** **cells** *in vitro.* **Effect of everolimus added** *in vitro* on cultured **cells.** Cells were treated with everolimus [100nM] for 2 and 24 hours. Cellular extracts were analyzed by western blot using the indicated antibodies. The graphs show the ratio between phosphorylated and total protein in each condition, expressed as fold induction versus control arbitrarily set at 1.

**Supplementary Figure S3. *In vivo* effects of TAK-228 on the T24 xenograft model.** 20.106 T24 cells, mixed with matrigel, were inoculated in a mouse to form a “donor” mass. The tumor was then excised and a single tumor fragment was surgically subcutaneously implanted into new mice (one implant/mouse). Treatment was administered when the average tumor volume reached approximately 200mm3. TAK-228 was tested in a continuous low dose (1mg/kg daily). Control mice were left untreated until the end of the experiment. A plot of the average tumor volume as a function of time in each treatment group is shown. \*p<0.05.

**Supplementary Figure S4. Cellular effects of TAK-117 on human bladder cancer** **cell lines *in vitro* and *in vivo.* A. Long-term *in vitro* exposure of tumor cells to TAK-117.** The indicated cells were plated at 1.5 x 103 to 5x103 cells/well (based on optimal density for each cell line) in 6-well plates and allowed to adhere overnight. The next day, cells were left untreated or treated with TAK-117 [3μM]. Media and drugs were replenished every 3 days. After 8-10 days, cells were fixed and stained with 0.06% crystal violet. Images of each plate were scanned and quantified. The intensity of blue was measured in arbitrary units using ImageJ. The number of viable cells in each treatment was plotted as a percentage of the control. Representative images of two independent experiments are shown. **B. Short-term sensitivity of BC tumor cells to TAK-117.** Cells were treated with escalating doses of TAK-117 (1, 3, 5, 10, 15, 20, 25µM) during 72 hours and cell viability was analysed by MTS assay. Shown values are the mean percentage +/- SD of cell viability relative to controls and plotted as dose-response curves using GraphPad Prism. IC50 values of TAK-117 for the 6 cell lines. Incorporates data from 3 replicate experiments.**C.** ***In vivo* exposure of subcutaneous CAL-29 xenografts to TAK-117.** CAL-29 tumor-bearingmice (n=6) were treated for 52 days with TAK-117 at 140mg/kg 3 consecutive days per week. Control mice (n=6) were left untreated until the end of the experiment. A plot of average tumor volume as a function of time in each treatment group is shown.

**Supplementary Figure S5. Effects of TAK-228 in combination with TAK-117 in RT4 cells.**

**A.** Effects of **TAK-228plusTAK-117** on cell cycle distribution. RT4 cells were treated with TAK-228 and TAK-117at the corresponding IC50 values (24.3nM and 15.7μM, respectively), or the combination of the 2 drugs for 24 hours. Fixed and propidium iodide stained cells were analyzed by flow cytometry. The bar chart shows the percentage of cells at different cell stages. **B.** Effects of **TAK-228plusTAK-117** on markers of the PI3K/mTOR pathway. The graphs correspond to the quantitative analysis of 3 replicates of the image of the western blot shown in Figure 5B. The graphs show the ratio between phosphorylated and total protein in each condition expressed as fold induction versus control arbitrarily set at 1. **C.** Effects of **TAK-228plusTAK-117** on autophagy. Cells were treated at the corresponding IC50 values for each drug, the combination of the 2 drugs, or everolimus [100nM], in the presence or absence of chloroquine (CLQ) [5μM], for 24 hours.The indicated autophagic markers were analysed in cell lysates by western blot and densitometric analysis form there replicates is shown in the graphs.

**Supplementary Figure S6. Effects of TAK-228 in combination with paclitaxel on bladder cancer models. A.** **Effects of TAK-228 in combination with paclitaxel on the PI3K/mTOR pathway *in vitro*.** RT4 cells were treated separately with TAK-228 and paclitaxel at the corresponding IC50 values, as well as with the combination of both drugs, for 2 and 24 hours. Cellular extracts were analyzed by western blot using the indicated antibodies. **B.** Effects of **TAK-228combination with paclitaxel** on cell cycle distribution. RT4 cells were treated with TAK-228 and paclitaxel (PAC) at the corresponding IC50 values (24.3nM and 5.3nM, respectively),or the combination of both drugs for 24 hours. Fixed and propidium iodide stained cellswere analyzed by flow cytometry.The bar chart shows the percentage of cells at different cell stages. **C.** Effects of **TAK-228combination with paclitaxel** on autophagy. Cells were treated at the corresponding IC50 values for each drug or the combination of both drugs in the presence or absence of chloroquine (CLQ) [5μM], for 24 hours. The indicated autophagic markers were analysed in cell lysates by western blot and densitometric analysis from there replicates is shown in the graphs. **D. Effects of TAK-228 in combination with paclitaxel on apoptosis.** Representative flow cytometry dot plots of 2 independent experiments are shown after simultaneously treating the cells with TAk-228 and paclitaxel at the corresponding IC50 values or the combination. Cells were processed as in Supplementary Figure S1A.